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Cerebral White Matter Hyperintense Lesions are Associated with Unstable Carotid Plaques

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Objectives. *The aim of this study was to determine whether unstable carotid plaques, a known risk factor for cerebral emboli, are associated with cerebral white matter lesions.*
Methods. *Seventy-one symptomatic patients undergoing magnetic resonance imaging prior to carotid endarterectomy for high grade carotid stenosis were included in this study. The number and volume of white matter hyperintense lesions (WMHL) on fluid attenuated inversion recovery brain scans were compared according to the morphology of carotid plaque based upon the American Heart Association (AHA) histological classification.*
Results. *Of the 57 patients who had good quality brain scans and non-fragmented carotid plaques, 15 plaques were defined as stable (type V) and 42 as unstable (type VI).*
After adjustment for the major risk factors affecting WMHL, unstable carotid plaques were found to be associated with more WMHL in the ipsilateral cerebral hemisphere than stable plaques (transformed means 2.50±1.2 vs. 1.53±1.1, $p = 0.016$*), however, there was only a trend towards larger WMHL volumes* ($p = 0.079$).
Conclusions. *The observed association between unstable carotid plaques and the number of white matter lesions suggest that thromboembolic plaque activity may contribute to the development of leukoaraiosis, in particular smaller individual lesions. Larger studies are warranted to confirm this finding and explore the potential clinical impact for selecting candidates for carotid endarterectomy.*

Keywords: Carotid plaque; White matter disease; Magnetic resonance imaging; Cerebrovascular disease.

Introduction

Leukoaraiosis is an abnormal CT appearance of the cerebral white matter in which there are hypodense, often poorly delineated, lesions.[1,2] On magnetic resonance imaging (MRI) leukoaraiosis is more conspicuous and seen as T2 weighted hyperintense white matter lesions (WMHL). WMHL are frequently seen in older people with and without neurological disorders and were long perceived to be non-specific. Large scale studies have, however, shown that WMHL are not only associated with increasing age, but also with arterial hypertension, diabetes and other vascular risk factors.[3] Moreover, the clinical relevance of WMHL has now been established with WMHL being more common in patients with a history of stroke, cognitive impairment and dementia, vascular parkinsonism, and depression,[3–6] and generally being associated with poor physical health and poor motor function.[7] Large population studies have shown WMHL to be predictive of stroke[6,8] and are thought to be an intermediate surrogate of stroke.[2] Identification and control of non genetic, non age-dependent factors leading to the development of WMHL is, therefore, of great interest as it may pave the way to establish interventions to reduce the risk of stroke or progressive cognitive impairment, mood or movement disorder thought to be associated with advanced WMHL.

Radio-pathological correlation studies reveal that punctuate and confluent WMHL tend to reflect varying degrees of cerebral ischaemia with a reduction in myelin, axonal loss, and astrocytic gliosis.[9–12] Pathologically, these changes have been termed

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selective incomplete white matter infarction and are separate from lacunar infarcts which are considered to be small strokes with imaging appearances of focal tissue destruction on T1 weighted scans.[1,2]

The possibility that WMHL have a micro-embolic aetiology is one explanation of the findings of epidemiological studies.[13–18] In patients with internal carotid artery disease, the presence of WMHL predicts stroke recurrence[13] and progression of WMHL is associated with increased incidence of stroke.[14] This implies that WHML and stroke share at least some risk factors. The aim of the study was to investigate the relationship between WMHL and carotid plaque morphology as surrogate marker of microembolic activity.[19] We hypothesized that if microembolism contributes to WMHL, they should be more frequent and extensive in the cerebral hemispheres ipsilateral to unstable carotid plaques compared to cerebral hemispheres ipsilateral to stable carotid plaques.

Methods

We report here on patients who had successfully undergone magnetic resonance imaging (MRI) of the brain and in whom a non-fragmented carotid endarterectomy specimen was available out of a cohort of 71 patients with a symptomatic high grade internal carotid stenosis. These patients had been prospectively and consecutively recruited for MR scanning prior to carotid endarterectomy as part of previously published studies[20] between May 1999 and June 2002. They were recruited from the fast track transient ischaemic attack (TIA) clinic and their symptoms included amaurosis fugax, transient ischaemic attacks or stroke within the previous 6 months. The severity of ipsilateral carotid stenosis on Duplex imaging was graded as 60–69%, 70–79%, 80–95% and 96–99% using the European Carotid Surgery Trial (ECST) criteria. Informed consent was obtained from all the patients and the study protocol was approved by the Local Research Ethics Committee.

After the carotid endarterectomy, the carotid plaques were collected and fixed in 10% formalin. Each specimen was then sectioned transversely (perpendicular to the lumen) into 5 mm blocks, starting from the specimen base, and then progressing distally until the whole specimen (including the bifurcation) was cut (Fig. 1). After embedding the blocks in paraffin, they were serially sectioned at 4 μm onto slides and then subsequently stained with Gill's hematoxylin and eosin 1%.

Each specimen was then viewed at ×4 to ×20 magnifications and classified using the American Heart Association (AHA) classification.[21] The unstable (type VI) plaque was diagnosed if any of the following features were present in any of the sections: free red blood cells within the intima and media not associated with the blood vessel lumen; organized lamellar plaque or luminal adherent thrombus; hemosiderin containing macrophages; surface defects or rupture. All of the specimens were either unstable (type VI) or stable (type V) (Fig. 2).

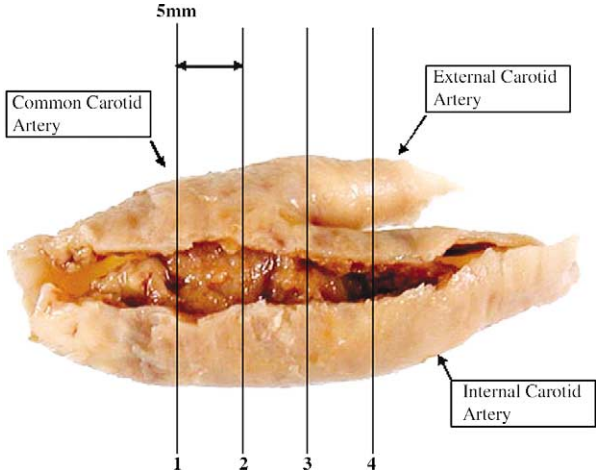

Fig. 1. Endarterectomy specimen. The whole specimen was cut in 5 mm intervals starting from the base of the specimen and then progressing distally, including the bifurcation of the common carotid artery.

Preoperative MRI studies were performed on a 1.5 T clinical scanner (Vision, Siemens Medical, Erlangen, Germany). To sensitively distinguish WMHL from similarly appearing lacunar infarcts, a fluid-attenuated inversion recovery (FLAIR) sequence was deployed (TR 9000 ms, TE 110 ms, TI 2500 ms, FOV 180×240 mm, 176×256, 4 mm slice

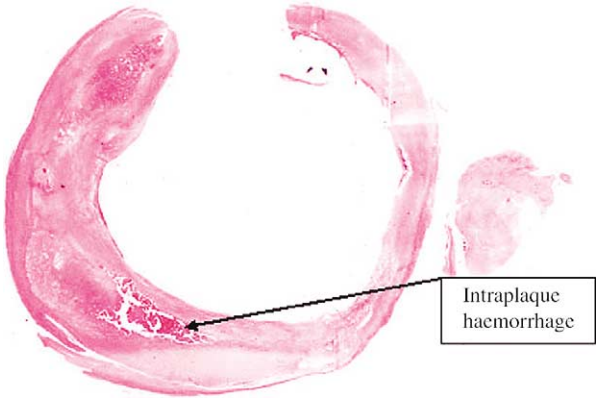

Fig. 2. Endarterectomy specimen. Microscopic views demonstrating intraplaque haemorrhage and the complicated carotid (AHA VI) plaque.

Fig. 3. Definition of WMHL (red outline) and lacunes (blue outline) in the right cerebral hemisphere using JIM software on axial FLAIR images.

thickness, 2 mm gap and two averages with subtotal brain coverage).

FLAIR images were processed off-line on UNIX workstations. Analysis was carried out by trained researchers using a semi-automated analysis program, [22,23] (Fig. 3) and blinded to the AHA status. Subcortical lesion findings were separated based on signal intensity on FLAIR into white matter hyperintense lesions (WHML) and lacunes. All peripheral and deep white matter lesions as well as periventricular lesions were included if ≥ 3 mm in diameter. Subcortical extensions from cortical infarcts were excluded.

Lesions were manually outlined in each of the 15 FLAIR axial slices, and summed per hemisphere to give a total hemispheric number of WHML (Fig. 3). Respective lesion volumes were automatically calculated. To correct for inter-individual variation in brain size, in particular in view of the unequal gender distribution that we subsequently observed between the stable and unstable plaque groups, the total intracranial volume (TICV) was manually outlined and ratios of the respective hemispheric lesion volumes to TICV are referred to as normalized lesion volumes. For group comparison, the hemispheric total number of WMHL and the normalized WMHL were calculated.

Statistical calculations were performed using the Statistical Package for Social Sciences (SPSS 11.0) software. Inter- and intra-observer variability for WMHL was confirmed by an assessment of 10 patients using the intraclass correlation coefficient. The number and normalised volume of ipsilateral WHML lesions in those with type V and type VI lesions were compared using the Mann–Whitney *U*-test. The main and consistent factors to affect the extent of WMHL are age, hypertension and a history of stroke.[1,2] To control for these factors, a multiple regression model was used to investigate the relationship of WMHL and carotid plaque morphology. The square root transformation was applied to the WMHL number and a log transformation to the WMHL volume in order to meet the assumptions of normality.

Results

Of the 71 patients who were recruited and underwent a carotid endarterectomy, 57 had non-fragmented carotid plaques and adequate FLAIR images, which were then subsequently analyzed (Fig. 4). The median time from the onset of symptoms to scanning was 13.7 weeks (inter-quartile range 9–22 weeks) and the median time from scanning to the carotid endarterectomy was 4 weeks (IQR 1–7 weeks).

Histology classified 15 carotid plaques (26.3%) as AHA type V stable plaque and 42 (73.7%) as AHA type V unstable plaque. Despite the small sample size, there were no significant differences in age, sex and other vascular risk factors between the AHA groups (Table 1). There was no association between the type

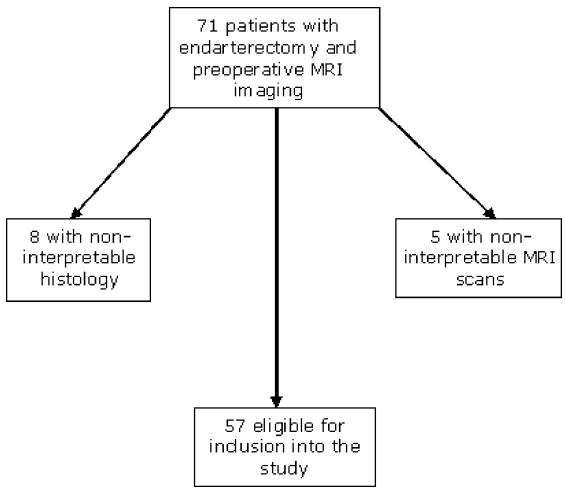

Fig. 4. Recruitment of the study population.

Table 1. Demographic breakdown of patients with the histologically complicated (AHA VI) and non complicated plaque (AHA V)

Variable	AHA VI plaque, ($n=42$)	AHA V plaque, ($n=15$)	p value
Age, year, mean ± SD	69.8 ± 8.6	67.6 ± 8.2	0.9
Sex—female, *n* (%)	17 (40)	11 (73)	0.08
Hypertension, *n* (%)	25 (59)	10 (64)	0.45
Smokers, *n* (%)	9 (21)	6 (40)	0.16
Statin treatment, *n* (%)	15 (36)	5 (33)	0.78
Diabetes mellitus, *n* (%)	9 (21)	3 (20)	0.8
Ischaemic heart disease, *n* (%)	13 (31)	6 (40)	0.7
Peripheral vascular disease, *n* (%)	11 (26)	2 (13)	0.3
Stroke, *n* (%)	9 (21)	8 (53)	0.2
TIA, *n* (%)	22 (52)	4 (27)	0.35
Amaurosis fugax, *n* (%)	11 (26)	3 (20)	0.6

There were no significant differences ($p>0.05$).

of symptom and the histological assessment of the carotid plaque.

There was good agreement between the two observers with regards to the WMHL number and volume (intra-class correlation coefficient =0.96 and 0.89, respectively). There was additionally good intra-observer correlation with regards to WMHL number and volume (intra-class correlation coefficient =0.98 and 0.90, respectively).

The median number of ipsilateral WHML in those with type VI lesions was 6.5 (IQR 3–12) compared to a median of 3 (IQR 0–6) (Mann–Whitney *U*-test $p=0.01$). The median normalized volume of ipsilateral WHML in those with type VI lesions was 1.15×10^{-3} (IQR 0.47–2.80) compared to a median of 0.42×10^{-3} (IQR 0.00–1.64) (Mann–Whitney *U*-test $p=0.11$).

The mean square root transformed WMHL number was 0.93 greater in the ipsilateral cerebral hemispheres of the patients with unstable carotid plaque than for stable carotid plaque (95% CI 0.18–1.67, $p=0.016$) after adjustment for age, hypertension and a history of stroke ($R=0.17$, Table 2). After adjustment for the above risk factors, a borderline significant difference ($R=0.19$, $p=0.079$) emerged between the log transformed, normalised WMHL volumes with type VI having larger WMHL volume between the two groups (regression coefficient =0.78, 95% CI −0.94 to 1.65, Table 2).

Discussion

This study investigated the relationship between cerebral white matter hyperintense lesions and carotid artery plaque morphology. Type VI carotid plaques, which are understood to be the type of plaque associated with macro and micro thromboembolism, were associated on average with over twice as many WMHL in the ipsilateral hemisphere compared with type V, stable plaques. The volume of these lesions was not different between those with different types of carotid plaque, but a borderline significantly larger volume was noted for type VI plaques after age adjustment. These findings suggest that microembolism may contribute to the development of WMHL and in particular to the development of small isolated lesions. This is in line with the known multi-factorial origin of WMHL with age and genetic factors the probable main determinants, but also non-ischaemic factors at play [1,2] such that the expected individual variability in lesion volumes will have reduced the statistical power in this relatively small sample. Interestingly, the number of WMHL was still sensitive enough with and without age adjustment to show an association with microembolic active plaques. This may be explained by the fact that the particle size and local haemodynamic factors are likely to determine the size of a WMHL resulting from a microembolic event whereas the number of WMHL should better reflect the number of relevant microembolic events.

Our observations provide evidence of a connection between carotid plaque morphology and WMHL or leukoaraiosis. This finding, as well as the lack of association between the degree of carotid artery stenosis and WMHL, is in line with previous research.[20,24] Several factors of carotid artery disease other than the degree of stenosis have been identified to predict subsequent thromboembolism and stroke.[19]

Table 2. WMHL number and normalized volumes of the ipsilateral hemispheres in patients ($n=57$) with the histological defined plaques (mean (± SD))

	Histological classification		p value*
	AHA VI, ($n=42$)	AHA V, ($n=15$)	
Transformed mean WMHL number† ± SD	2.50 (±1.2)	1.53 (±1.1)	0.016
Transformed normalized WMHL volume‡ ± SD	−3.2 (±1.2)	−4.13 (±1.9)	0.079

A linear regression model was used and the transformed means are given.
* Adjusted for age, hypertension and a history of stroke.
† WMHL number transformed using square root transformation.
‡ Normalized WMHL volume transformed using log transformation.

Type VI plaque, with its surface ulceration, rupture and haemorrhage, is the type most likely to cause platelet aggregation and thrombosis over the plaque and hence, cause cerebrovascular events.[21] Our findings suggest that some WMHL too may have a direct thromboembolic aetiology. Several studies have demonstrated that the features associated with the type VI plaque are not only associated with increased emboli as detected by clinical symptoms[25–27] but also with transcranial Doppler (TCD) detection of microembolic signals[24,28]—the latter being clinically silent. These observations indicate that sub-clinical microembolic mechanisms exist, and could provide a rationale for how unstable plaques could cause WMHL.

The strength of the present study relate to the uniform histological classification of plaques and quantitative MRI analysis. The main limitation is, however, the small sample size with the risk of confounding unbalanced vascular risk factors between groups. There were, however, no apparent differences in symptoms, vascular risk factors or treatment between the groups. Another limitation relates to our inability to subdivide the location of WMHL into periventricular, paraventricular deep and subcortical white matter, which are thought to differ in their likely aetiology.[16] As a result we may have obscured a more highly specific association between carotid disease and a WMHL subtype. Lastly, the observed association between carotid disease and WMHL may be an indirect one as carotid arteriosclerosis could be a marker of small vessel disease.

The potential clinical impact of the findings relate directly to the individual assessment of surgical risk *vs.* potential benefit. First, the proposed microembolic source of WMHL offers an explanation why patients with more WHML are at higher risk of operative complications during carotid endarterectomy,[14] and may thus, prompt alternative surgical strategies in those individuals with large numbers of WMHL and plaque imaging characteristics suggestive of an unstable plaque on preoperative work-up. Second, the confirmation of the proposed thromboembolic aetiology of WMHL in a specific subtype of carotid plaque would provide the rationale for studies to test the potential benefits of CEA beyond stroke prevention, namely to prevent the progression of leukoaraiosis associated conditions such as vascular cognitive impairment.[1,2]

In conclusion, this study provides evidence that microemboli associated with unstable carotid plaques may cause WMHL. Due to the relatively small sample size independent replication in a larger sample is warranted. This could be based on a MRI surrogate for the unstable plaque which would further allow performing interhemispheric comparison as a means to directly control for individual risk factors such as vulnerable genes and the overall extent of large and small vessel arteriosclerosis in an individual.

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There are no conflicts of interests in this study.

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